

CLAIMS

We claim:

1. A method for producing an immunoglobulin exhibiting a
5 higher affinity for an antigen, comprising the steps of:

10 introducing at least one mutation into a parent polynucleotide sequence encoding an immunoglobulin chain variable region to produce a mutant sequence, wherein said mutant sequence encodes a variable region that has a different pattern of glycosylation sites than a variable region encoded by said parent polynucleotide sequence; and

15 expressing said mutant sequence in a cell.

2. The method of Claim 1, wherein said mutant sequence has at least one mutation in a V region framework.

20 3. The method of Claim 2, wherein the mutant sequence encodes a variable region that has fewer glycosylation sites than the variable region encoded by the parent polynucleotide sequence.

4. The method of Claim 3, wherein said mutant sequence encodes a variable region that has no glycosylation sites and the variable region encoded by the parent polynucleotide sequence has at least one glycosylation site.

25 5. The method of Claim 1, wherein the mutation is a substitution mutation that changes at least one codon of the parent polynucleotide sequence to a different codon at the same position in the mutant sequence.

30 6. The method of Claim 5, wherein the substitution mutation occurs in a consensus N-linked glycosylation site sequence present in the parent polynucleotide sequence, said site selected from the group consisting of:

(1) -Asn-X-Ser-; and

(2) -Asn-X-Thr-;

where X may be any conventional amino acid, other than Pro.

5 7. The method of Claim 6, wherein the substitution mutation results in a conservative amino acid substitution.

10 8. The method of Claim 1, wherein the V region framework is substantially identical to a V region framework of a heavy chain variable region.

15 9. The method of Claim 8, wherein the V region framework is substantially identical to a V region framework of a human heavy chain variable region.

20 10. The method of Claim 8, wherein said heavy chain variable region comprises a V region framework substantially identical to a V region framework of a first species and at least one complementarity determining region substantially identical to a second species.

25 11. A method of Claim 8, wherein the V region framework is substantially identical to an amino acid sequence selected from the group consisting of:

-Lys-Ala-Thr-Leu-Thr-Val-Asp-Asn-Ser-Ser-Ser-Thr-Ala-Tyr-; and

-Lys-Ala-Thr-Ile-Thr-Ala-Asp-Glu-Ser-Thr-Asn-Thr-Ala-Tyr-.

30 12. The method of Claim 10, wherein the V region framework is substantially identical to murine M195 heavy chain V region framework.

35 13. The method of Claim 10, wherein the V region framework is substantially identical to V region framework of humanized M195 heavy chain.

14. A method for increasing affinity of an antibody for an antigen, comprising the steps of:

5 producing a mutation that removes a glycosylation site in a variable region of a parent immunoglobulin chain to produce a glycosylation-reduced immunoglobulin; and,

10 expressing said glycosylation-reduced immunoglobulin in a cell.

15. The method of Claim 14, wherein the mutation removes a consensus N-linked glycosylation site sequence.

15 16. The method of Claim 14, wherein the mutation removes a glycosylation site in a V region framework.

20 17. A method for producing a glycosylation-supplemented immunoglobulin, comprising the steps of:

20 introducing a mutation into a parent sequence, wherein the mutation creates a consensus N-linked glycosylation site sequence, said site selected from the group consisting of:

- (1) -Asn-X-Ser-; and
- (2) -Asn-X-Thr-;

25 where X may be any conventional amino acid, other than Pro.

30 18. A mutant immunoglobulin, comprising at least one immunoglobulin chain having a V region framework wherein at least one naturally-occurring glycosylation site that is present in a parent immunoglobulin sequence is abolished in the mutant sequence, and wherein the mutant immunoglobulin has an affinity for antigen that is higher than the parent immunoglobulin.

35 19. A mutant immunoglobulin of Claim 18, wherein the mutant immunoglobulin has at least four-fold higher affinity for antigen than the parent immunoglobulin.

20. A mutant immunoglobulin of Claim 18, wherein at least one carbohydrate moiety is attached to a constant region amino acid residue through N-linked or O-linked glycosylation.

5 21. A mutant immunoglobulin of Claim 18, wherein said naturally-occurring glycosylation site is present in the parent immunoglobulin in a region spanning from about amino acid residue 65 to about amino acid residue 85.

10 22. A mutant immunoglobulin of Claim 18, wherein said naturally-occurring glycosylation site is present in the parent immunoglobulin in a region adjacent to a CDR.

15 23. A mutant immunoglobulin, comprising at least one immunoglobulin chain having a glycosylation site at a position in a V region framework, wherein said glycosylation site is not present in a naturally-occurring V region framework at said position in a parent sequence.

20 24. A mutant immunoglobulin according to Claim 23, wherein the glycosylation site is in a V region framework.

25 25. A glycosylation-reduced antibody having a higher affinity than a parent antibody.

26. A glycosylation-supplemented antibody.

27. A polynucleotide comprising a nucleotide sequence that encodes a mutant immunoglobulin.

30 28. A cell containing a polynucleotide of Claim 27.

29. A composition comprising at least one mutant immunoglobulin.